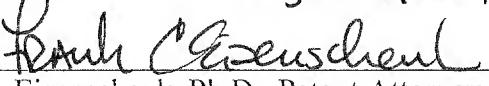


I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on August 12, 2009.

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. BKR.107


Frank C. Eisenschenk, Ph.D., Patent Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Dominique Lombardo, Eric Mas, Marie-Odile Sadoulet, Laurence Panicot-Dubois, Jean-Paul Bernard
Issued : July 7, 2009
Patent No. : 7,557,193
Conf. No. : 2965
For : Glycopeptides Derived From Pancreatic Structures, Antibodies and Applications Thereof in Diagnostics and Therapeutics

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:

Column 17, line 36:

“ma16D10”

Application Reads:

Page 22, line 30:

--mAb16D10--

Column 18, line 57:

“1089 Phe364”

Page 24, lines 20-21:

--1089: Phe364--

Column 20, line 13:

“B) Bacterial Transformation”

Page 26, line 10:

--b) Bacterial Transformation--

Column 20, line 26:

“C) Plasmid Purification”

Page 26, line 21:

--c) Plasmid Purification--

Column 25, line 34:

“NaCl/50 µl”

Page 33, line 11:

--NaCl/150 µl--

Column 27, line 9:

“manner pancreatic”

Pages 34-35, lines 36-1:

--manner: pancreatic--

Column 27, line 22:

“prepared”

Pages 35, line 9:

--prepared:--

Patent Reads:

Column 62, lines 34-35:

“antibody is 16D10 is humanized”

Application Should Read:

Notice of Allowance dated March 23, 2009
(original claim 74, renumbered as claim 20):

--antibody 16D10 is humanized--

Column 62, lines 37-38:

“derivative or monoclonal antibody is 16D10
is chimeric”

Notice of Allowance dated March 23, 2009
(original claim 75, renumbered as claim 21):

--derivative of monoclonal antibody 16D10 is
chimeric--.

A true and correct copy of pages 22, 24, 26, 33, 34, and 35 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

The Commissioner is also authorized to charge any additional fees as required under 37 CFR 1.20(a) to Deposit Account No. 19-0065.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/jb

Attachments: Copy of pages 22, 24, 26, 33, 34, and 35 of the specification

particular, the invention relates to the use of an antibody according to the invention or of a pharmaceutical composition comprising such antibody for preparing a medicament intended for the preventive or curative treatment of a pancreatic pathology, preferably pancreatic cancer, or breast cancer. Preferably, the antibody is the antibody 16D10, a fragment or derivative thereof, or an antibody which essentially binds to the same epitope or determinant as the former. The antibody can also be the antibody J28, a fragment or derivative thereof, or an antibody which essentially binds to the same epitope or determinant as the former. In a particular embodiment, said antibody is coupled with an antitumoral substance.

10 The invention relates to a method of preventive or curative treatment of a subject suffering from a pancreatic pathology, in particular pancreatic cancer, or breast cancer, comprising administering to said subject an effective amount of an antibody according to the invention, said administration resulting in a decrease or disappearance of the pancreatic pathology, in particular pancreatic cancer, or breast cancer, in the subject.
15 Preferably, the antibody is the antibody 16D10, a fragment or derivative thereof, or an antibody which essentially binds to the same epitope or determinant as the former. The antibody can also be the antibody J28, a fragment or derivative thereof, or an antibody which essentially binds to the same epitope or determinant as the former. In a particular embodiment, said antibody is coupled with an antitumoral substance.

20 In another protocol the usual dose, which varies according to the subject being treated and the causal disease, can be for example from 1 to 10 mg of the monoclonal antibody described in example 18 hereinbelow per kilogram of body weight administered systemically in humans, once a week for two weeks.

25 The invention is also directed at providing new products (glycopeptides, antibodies, etc.) as well as other better, more effective or purer products than those described in the prior art.

The invention is illustrated in the following examples.

Legends of figures

30 **Figure 1.** Study comparing the reactivity of mAbJ28 and mAb16D10 in two pancreatic tumor tissues (PDAC = ADK1 and PDAC4 = ADK4).

Figure 2. Competition test between antibodies mAbJ28 and mAb16D10 for glycopeptide J28.

35 **Figure 3.** Competition test between antibodies mAbJ28 and mAb16D10 in SOJ-6 cells.

Figure 4. Comparative immunodetection analysis in urine from healthy

amplification of nucleotide sequences particularly rich in guanine and cytosine. The following program was employed : one cycle of 2 minutes at 94°C followed by 35 cycles of 1 minute at 94°C (denaturation), 1 minute at 52°C (primer annealing), and 4 minutes at 68°C (extension), followed by one cycle of 10 minutes at 68°C, in a Robocycler Gradient 5 96 thermocycler (Stratagene).

The primers had the following sequences :

N-ter/FAPP (SEQ ID No 1) :

5'- TTCGTAaggcttGCGAAGCTGGCGCCGTACAGAA-3';

C-ter/FAPP-BSDL (SEQ ID No 2) :

10 5'-TTTCGTTaaattcACGCTAAAACCTAATGACTGCAGGCATCTG-3'.

The C-ter/FAPP-BSDL primer (SEQ ID No 2) which was used comprises a termination codon so as to eliminate translation of the c-myc epitope as well as that of the 6x-histidine tag carried by the commercial vector.

15 The cDNA (nucleotides 1 to 2169) amplified in this manner (SEQ ID No 12) does not comprise a signal peptide.

The resulting cDNA was then cloned into plasmid pSec-Tag (Invitrogen, Leek, the Netherlands).

Preparation 2 : Plasmid pSec-16R

20 The cDNA coding for the C-terminal part of BSDL (from nucleotide 1089 : Phe364 to nucleotide 2169 : stop codon; SEQ ID Nos 8 and 9) from RNA extracted from normal pancreas was amplified by PCR using the primers C-ter/FAPP-BSDL (primer SEQ ID No 2, see Preparation 1) and N-ter-Ct.

The N-ter-Ct primer had the following sequence :

25 N-ter-Ct (SEQ ID No 3) :

5'- CGTCTAaaggcttTTTGATGTCTACACCGAGTCC-3'.

The polymerase chain reaction was carried out in the conditions described earlier (see Preparation 1).

The resulting cDNA was then cloned into plasmid pSec-Tag.

30

Preparation 3 : Plasmid pSec-6R

The cDNA coding for the C-terminal part of FAPP (from nucleotide 1089 : Phe364 to nucleotide 1839 : stop codon; SEQ ID Nos 12 and 13) present in the plasmid pSec-FAPP was amplified by PCR using nucleotide primers No 2 (C-ter/FAPP-BSDL) and 35 No 3 (N-ter-Ct) described hereinabove.

The polymerase chain reaction was carried out in the conditions described

the C-terminal part of FAPP, obtained by PCR, was directly cloned after purification and *Hind*III/*Eco*RI digestion into the pSec-Tag plasmid to yield plasmid pSec-6R (6R for C-terminal of domain FAPP) (SEQ ID No 12). Sequencing was carried out with the universal T7 primer and the BGH reverse primer synthesized for this purpose by Euro Sequences

5 Gènes Service.

The PCR product coding for α (1-3) galactosyltransferase was released with the restriction enzymes *Eco*RI and *Apal*, purified, then directly ligated into the *Eco*RI/*Apal* sites of the vector pBK-CMV to yield plasmid pBK- α GT.

10 **b) Bacterial transformation**

A 5 μ l aliquot of the ligation product was contacted with 50 μ l of competent bacterial cells (*Escherichia coli*, strain TOP10F') according to the protocol described by Hanahan [Hanahan, 1983, J. Mol. Biol., 166: 557-580]. Two microliters of 0.5 M β -mercaptoethanol were added and the sample was kept on ice for 30 minutes, then heat-15 shocked at 42°C for 30 seconds, and immediately put back on ice. After 2 minutes, the sample was diluted in 450 μ l of SOC medium (Life Technologies). The bacterial suspension was incubated for 1 hour at 37°C with shaking. The bacteria were then spread, using glass beads, on Petri dishes containing Luria-Bertagni agar supplemented with 50 μ g/ml ampicillin.

20

c) Plasmid purification

The bacterial colonies which appeared after 18 hours of culture on selective agar medium (50 μ g/ml ampicillin) were picked and inoculated in 2 ml of Luria-Bertagni liquid medium containing 50 μ g/ml ampicillin. The cultures were incubated for 8 hours at 25 37°C with shaking, then 1.5 ml of the bacterial suspension was centrifuged at 5,000 g for 5 minutes. The bacterial pellet was taken up in 100 μ l of bacterial membrane destabilizing buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, Ribonuclease A 100 μ g/ml). The bacterial suspension was lysed by addition of 200 μ l of alkaline lysis buffer (200 mM NaOH, 1% SDS) and the pH of the preparation was neutralized with 150 μ l of 3 M potassium acetate 30 pH 5.5. The alkaline lysis and neutralization steps require a 5-minute incubation on ice. Cell debris, denatured proteins and chromosomal DNA were eliminated by centrifugation at 10,000 g for 10 minutes at 4°C. The supernatant was then extracted with phenol-chloroform by 1:2 dilution in phenol/chloroform/ isoamyl alcohol (25/24/1 V/V/V) stabilized 35 to pH 8 with 100 mM Tris-HCl. The aqueous and organic phases were separated by centrifugation at 10,000 g for 2 minutes at room temperature. The upper aqueous phase was recovered and diluted 1:3 in absolute ethanol (-20°C). Plasmid DNA was precipitated

characteristics of those corresponding to the C-terminal end of FAPP were prepared as follows :

Stage A - Immunization of mice.

Six-week-old male Balb/c mice were immunized according to the following
5 protocol :

- Day 0 : Intraperitoneal injection of 25 µg of a mixture of FAPP and BSDL purified from normal and pathological human pancreatic juices (hereinbelow named BSDL-FAPP antigen) in a 50:50 emulsion (150 µl NaCl/150 µl complete Freund's adjuvant).
- 10 Day 15 : Intraperitoneal challenge with 25 µg of BSDL-FAPP antigen in a 50:50 emulsion (150 µl NaCl/150 µl incomplete Freund's adjuvant).
- Day 30 : Intraperitoneal challenge with 25 µg of BSDL-FAPP antigen in a 50:50 emulsion (150 µl NaCl/150 µl incomplete Freund's adjuvant).
- Day 110 : Intraperitoneal challenge with 20 µg of BSDL-FAPP antigen in a 50:50 emulsion (150 µl NaCl/150 µl incomplete Freund's adjuvant).
- 15 - Day 140 : Intraperitoneal challenge with 20 µg of BSDL-FAPP antigen in a 50:50 emulsion (150 µl NaCl/150 µl incomplete Freund's adjuvant).
- Day 215 : Intraperitoneal challenge with 20 µg of BSDL-FAPP antigen in a 50:50 emulsion (150 µl NaCl/150 µl incomplete Freund's adjuvant).
- 20 - Day 244 : Intravenous injection of 10 µg of BSDL-FAPP antigen in 100 µl of sterile NaCl.
- Day 247 : Cell fusion.

Stage B – Cell fusion according to the protocol of Köhler and Milstein.

- 25 a) At day 247, the selected mouse was sacrificed and the spleen removed and ground up. The spleen cells were washed in RPMI 1640 medium. P3.X63.Ag8 653 myeloma cells, previously grown in RPMI 1640 medium containing 20% fetal calf serum (FCS), 1% glutamine, 1% nonessential amino acids and 1% sodium pyruvate were also washed in the same medium.
- 30 At the same time, peritoneal macrophages were collected by peritoneal lavage of non-immunized Balb/c mice with RPMI. For hybridoma formation, the spleen cells and myeloma cells were mixed in a tube at a ratio of 5 spleen cells for 1 myeloma cell. After centrifugation, the cell pellet was resuspended in 800 µl of 50% polyethylene glycol 1500 in 75 mM Hepes buffer pH 7.5. After 1 minute of contact at 37°C, 20 ml of RPMI 1640 medium were slowly added to the fused cells.

b) The initial culture was carried out on 96-well microtitration plates in the presence of RPMI medium containing 20% fetal calf serum (FCS) and supplemented with 5×10^{-3} M hypoxanthine, 2×10^{-5} M aminopterin and 8×10^{-4} M thymidine. Next, 5×10^3 peritoneal macrophages followed by 10^5 fused cells were deposited in each well.

5

Stage C – Cloning and subcloning.

Each hybridoma selected by the method described in stage D hereinbelow resulted from cloning by a limit dilution technique in which 10, 5, 2, 1 and 0.5 cells were statistically distributed into microwells containing peritoneal macrophages. Two 10 subclonings were thus carried out, each clone and subclone having been replicated then frozen in 90% FCS and 10% dimethylsulfoxide (DMSO). Subclones from the last generation were then expanded *in vivo* to obtain ascites fluid in the Balb/c mice, followed by immunoglobulin purification on a protein A column.

15 Stage D – Hybrid cell selection method.

Selection was carried out by a liquid phase ELISA method using culture supernatant. Five micrograms of BSDL-FAPP antigen in 100 µl of bicarbonate buffer pH 8.5 were deposited in the wells of a 96-well ELISA plate. The plate was activated for 12 h at 4°C and saturated for 2 hours with 300 µl of 1 mg/ml bovine albumin. The plates were 20 then washed and incubated with 100 µl of cell culture supernatants potentially containing antibodies directed against the BSDL-FAPP antigen. After a 2-hour incubation, the plates were washed and incubated for 2 hours with alkaline phosphatase-labelled secondary antibodies. At the end of the incubation, the plates were again washed and incubated with para-nitrophenylphosphate (100 µl, 1 mg/ml in 0.2 M Tris/HCl buffer pH 8.2 and 1 mM 25 CaCl₂). After 1 hour at 37 °C, the plates were read on a microplate reader at 410 nm.

About 15 antibodies were selected for their response in the ELISA test. Additional analyses by Western blot (immuno-imprinting) using FAPP as immunogenic protein enabled the selection of hybridomas 7B4 (Example 14), 11D7 (Example 15), 14H9 (Example 16), 14H10 (Example 17), 16D10 (Example 18) and 8H8 (Example 19).

30 Cells producing the IgM antibody 16D10 were deposited with the Collection Nationale de Culture de Microorganismes (CNRM) in Paris on 16 March 2004 under the number I-3188.

Example 20. Preparation of membrane glycopeptides of natural origin

35 Membrane glycopeptides carrying epitopes recognized by the monoclonal antibodies described in examples 14 to 19 were prepared in the following manner :

pancreatic tumor cells were cultivated on a plastic support then detached therefrom with non-enzymatic dissociation solution (Sigma). The cell pellet obtained by centrifugation (2 min at 1000 rpm) was sonicated (2 x 15 sec) and again centrifuged (20 min, 14,000 rpm, 4°C). The pellet suspended in phosphate buffer corresponds to membrane glycopeptides 5 carrying epitopes recognized by the monoclonal antibodies described in examples 14 to 19.

Example 21.

A vaccine having the following composition was prepared :

10	- membrane glycopeptides from example 20 isolated from HaPT-1 pancreatic cells,	20 µg
	- ALU-gel-ser adjuvant (Serva)	150 µl
	- excipient including water for injections	150 µl

Vaccination was carried out by intraperitoneal injection two weeks then one 15 week before transplanting the tumor cells into the flank of the animals. The result of said vaccination was compared to that of a placebo (injection of isotonic solution).

Example 22.

A 0.5 mg/ml injectable isotonic solution of antibodies from example 18 20 (16D10) was prepared.

Control Example 1 : Recombinant glycopeptide having the size and the characteristics of those corresponding to the C-terminal end of BSDL

25 The method described in example 1 was employed, but by cultivating the cell clone CHO-K1.

The gel did not show any bands at molecular weight 78 kDa or 83 kDa.

30 PHARMACOLOGICAL STUDY

Experiment 1 : Use of recombinant C-terminal glycopeptides of BSDL and FAPP in cellular immunotherapy of exocrine pancreatic cancer

35 Operating protocol :

Hamsters (90-100 g) were divided into several groups and vaccinated with

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,557,193

Page 1 of 1

APPLICATION NO.: 10/593,859

DATED : July 7, 2009

INVENTORS : Dominique Lombardo, Eric Mas, Marie-Odile Sadoulet,
Laurence Panicot-Dubois, Jean-Paul Bernard

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 17,

Line 36, "ma16D10" should read --mAb16D10--.

Column 18,

Line 57, "1089 Phe364" should read --1089: Phe364--.

Column 20,

Line 13, "B) Bacterial Transformation" should read --b) Bacterial Transformation--.
Line 26, "C) Plasmid Purification" should read --c) Plasmid Purification--.

Column 25,

Line 34, "NaCl/50 µl" should read --NaCl/150 µl--.

Column 27,

Line 9, "1089 Phe364" should read --1089: Phe364--.

Line 22, "prepared" should read --prepared:--.

Column 62,

Lines 34-35, "antibody is 16D10 is humanized" should read
--antibody 16D10 is humanized--.

Lines 37-38, "derivative or monoclonal antibody is 16D10 is chimeric" should read
--derivative of monoclonal antibody 16D10 is chimeric--.

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